OPINION

A self-organization framework for symmetry breaking in the mammalian embryo

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Abstract | The mechanisms underlying the appearance of asymmetry between cells in the early embryo and consequently the specification of distinct cell lineages during mammalian development remain elusive. Recent experimental advances have revealed unexpected dynamics of and new complexity in this process. These findings can be integrated in a new unified framework that regards the early mammalian embryo as a self-organizing system.

In many classes of animals, such as insecta and amphibia, a framework for the body pattern is present in the zygote. A simple cue, such as the position of the egg within the ovary or the point at which a sperm enters the egg, induces asymmetry in the zygote, and this in turn controls cell positioning and specification of cell fate during the course of development. Mammalian development is distinct, because the embryo seems to maintain its full symmetry even after fertilization of the egg. Until the 8-cell stage at embryonic day 2.5, all cells in a mammalian embryo are indistinguishable in terms of geometry, developmental potential (reviewed in REFS 1, 2) and gene expression pattern[3]. During the following divisions, asymmetry between cells emerges as polar and apolar cells appear[4] and as these cells are positioned in the inner or the outer layers of the embryo[5,6]. This process of symmetry breaking concludes only two days later with the establishment of two distinct cell lineages in the blastocyst: the embryonic inner cell mass and the extra-embryonic trophectoderm. Thus, at embryonic day 4.5, these two cell types occupy defined spatial domains in the blastocyst and have distinct molecular signatures (BOX 1). Intriguingly, recent live-imaging studies showed that cells remain very dynamic as the symmetry of the embryo is broken[7–9], changing their position relative to each other at each cell division[10]. Division patterns are not stereotypic in space and time, and the lineage segregation process seems to be influenced by many interdependent factors, including cell polarity, cell–cell interactions, the activity of signal transduction pathways and the orientation of cell division planes. Furthermore, the early mammalian embryo has a highly regulative capacity, and cell fate remains reversible up to the blastocyst stage of development[11,12] (BOX 2).

This complexity has so far made it difficult to determine the principles underlying symmetry breaking and lineage segregation during mammalian blastocyst development, a process that is known as blastocyst patterning. Accordingly, several key issues regarding blastocyst patterning remain unresolved and relatively unexplored. First, we do not know how the initial asymmetry between cells arises, which leads to the formation of the inner cell mass and the trophectoderm. Another open question concerns the adjustment mechanism by which blastocyst patterning — which defines the outer trophectoderm and the inner cell mass — is reproducibly established from the process of symmetry breaking despite the high variability in terms of division pattern, positioning of individual cells and gene expression pattern.

Advances in molecular biology and microscopy in recent years have provided new insights into early mouse development but have also revealed unexpected complexities. We believe that our understanding of the symmetry breaking process will benefit from the integration of these findings into a new conceptual framework of early mammalian blastocyst patterning.

Here, we review three classic models of blastocyst patterning and lineage segregation in mouse embryos and present their key aspects. We discuss their limitations in view of the results obtained recently from new experimental methods. This leads us to propose a unifying framework to explain mammalian embryo patterning on the basis of self-organization. Finally, we discuss remaining key questions and possible approaches to answer these questions in the context of this self-organization framework. We hope that this will lead to a comprehensive understanding of symmetry breaking and lineage commitment during early mammalian development.

Classic models of lineage segregation

To explain the establishment of the first two cell lineages during mammalian preimplantation development, three models have been proposed in the past few decades (FIG. 1). These models are either derived from analogy to other model organisms or based on key findings from studies of mammalian development. Although they have not always been critically discussed, there are marked differences between these models in terms of the mechanism underlying the specification of individual cells.

The pre-patterning model. In analogy to other model organisms such as *Drosophila melanogaster*[13,14], *Caenorhabditis elegans*[15] and *Xenopus laevis*[16,17], the first conclusive model of mammalian blastocyst patterning involved molecular determinants that were postulated to be asymmetrically localized in the oocyte during maturation or upon fertilization[18]. In the cleavages that follow fertilization, these determinants would be differentially segregated between daughter cells, and lineage commitment would depend on the determinants that a daughter cell receives (FIG. 1a). This model has been recurrently proposed, including in recent publications. For example, the position at which a sperm enters the oocyte was proposed to have a key role in patterning the mouse embryo[19], and 2-cell[20,21] or 4-cell[22,23] blastomeres have been claimed to have distinct potencies in terms of their future fate, possibly owing to the inheritance of specific cytoplasmic components.

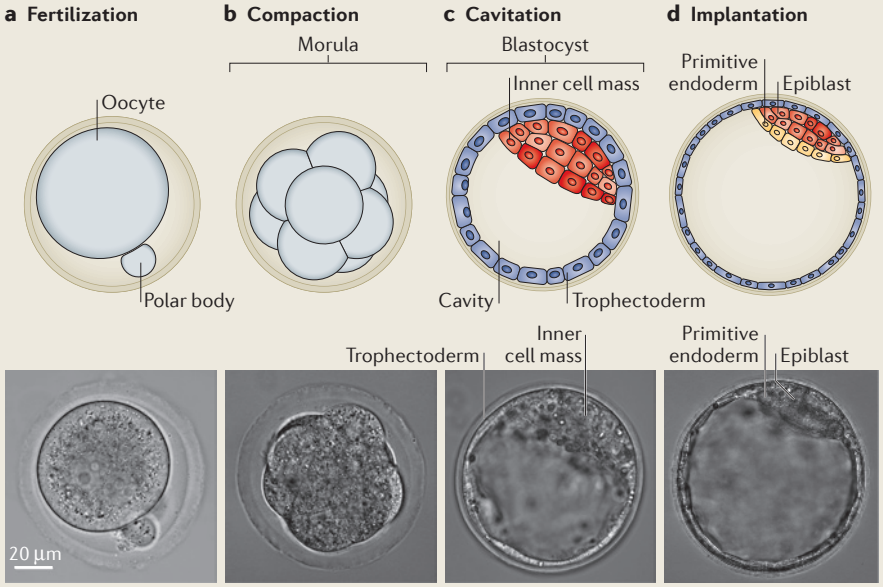
The decisive confirmation of this model would be the identification of a determinant that is asymmetrically localized in the zygote and that can drive lineage differentiation upon induction or ectopic application. Such a molecular determinant has not yet been identified, and although this is not sufficient to disprove the hypothesis, the pre-patterning model does therefore remain controversial[24–29]. Theoretical and modelling approaches, as discussed below, might show whether a localized determinant is required for the symmetry breaking process.

The inside–outside model. The early mammalian embryo has a surprising level of plasticity (BOX 2). When single blastomeres were isolated from the 2-cell, 4-cell or 8-cell stages of the embryo, they were able to develop

to term or to form mini-blastocysts. Thus, individual cells are still equivalent at the 8-cell stage and are not predetermined to a specific lineage by the molecular determinants they contain[30,31]. These findings are incompatible with the pre-patterning model, and they suggest a more flexible model of cell lineage specification, such as the inside–outside model proposed by Tarkowski and Wróblewska in 1967 (REF. 31) (FIG. 1b). In this model, the position of a cell in the morula (rather than any intrinsic factor) determines its fate. Outside cells become trophectoderm, whereas inside cells form the inner cell mass. Supporting this model, a recent study showed that Hippo signalling is involved in determining lineage commitment towards the trophectoderm or inner cell mass[32]. It was proposed that cell fate induction is triggered by a position-sensing mechanism within the embryo that lies upstream of the Hippo pathway. The identification of such a mechanism would be key for understanding and evaluating this model. However, recently it was discovered that cells in the morula exhibit molecular heterogeneity during the patterning process to early blastocysts[33–35]. This finding is not compatible with the inside–outside model, as such heterogeneity would not be generated between cells at equivalent positions by this model alone.

The cell polarity model. Several years after the inside–outside model was proposed, apical membrane domains on blastomeres isolated from the 8-cell stage embryo were identified in terms of their distinct distribution of microvilli and cell organelles[36] and the specific binding of lectins[37]. These findings were later confirmed by the localization of specific proteins with a known role in cell polarity[38,39]. The observation that these apical membrane domains are inherited during cell division led Johnson and Ziomek to propose the cell polarity model in 1981 (REF. 4) (FIG. 1c). After compaction at the 8-cell stage, depending on the orientation of the division plane, the newly polarized cells can transmit their apical domain either to both daughter cells or to only one daughter cell through symmetric or asymmetric division, respectively. This model posits that cells that inherit apical domains become trophectoderm, whereas other cells form the inner cell mass. Thus, this model introduced the relationship between the division plane and the transmission of apical domains as the key determinant for lineage selection. A recent study using mice entirely devoid of maternal and zygotic epithelial cadherin (E-cadherin) showed that individual cells with an apical domain can express the homeobox transcription factor and trophectoderm marker CDX2 in the absence of epithelial integrity, which suggests that the apical domains have a more important role in determining cell lineage differentiation than cell–cell interactions[40]. However, a causal link between the orientation of the division plane and cell fate specification *in vivo* remains to be demonstrated. As the orientation of the division axis is generally influenced by factors such as cell shape, cell–cell adhesion, epithelial polarity and cell rounding during mitosis[41], testing the cell polarity model will require evaluating the morphology of cells in addition to a read-out of cell differentiation, such as a lineage-specific gene marker. In any case, this model alone cannot explain the regulative nature of early mammalian development.

Box 1 | **Mouse pre-implantation development: from fertilized oocyte to blastocyst**



Fertilization
Embryonic development starts with fertilization, when a single sperm cell penetrates the cell membrane of the oocyte and delivers its haploid genome into the oocyte. This event triggers the oocyte to complete the second meiotic division, leading to the formation of a diploid cell (see the figure, part **a**). Subsequent mitotic divisions (known as cleavages) generate blastomeres without increasing the total cytoplasmic volume of the embryo.

Compaction
At the 8-cell stage, cell adhesion increases and blastomeres flatten up on each other. At the same time, cells also develop apico–basal polarity in an outside–inside orientation. This process is known as compaction and results in a spherical cell aggregate known as the morula (see the figure, part **b**). During the following two rounds of cell division, cells acquire for the first time differences in cell position and morphology, with polar cells lying on the outside and apolar cells filling up the inside of the morula.

Cavitation and blastocyst formation
Around the 16-cell to 32-cell stage of the morula, outer cells mature into an epithelium with tight junctions between cells and start to pump fluid from the outside into the inside of the embryo. The fluid accumulates in the intercellular spaces, which coalesce and form a cavity of gradually increasing size (see the figure, part **c**). The embryo is now called a blastocyst. Clustered on one side of this cavity is the pluripotent inner cell mass, and the epithelial outer cells form the trophectoderm.

Implantation
The mature blastocyst continues to pump fluid into the cavity and eventually makes contact with the uterus, which marks the start of implantation. Around the same time, the inner cell mass has completed a second lineage divergence into the primitive endoderm (which lines the cavity) and the pluripotent epiblast (which is surrounded by primitive endoderm and trophectoderm) (see the figure, part **d**). Only cells of the epiblast form the embryo proper, whereas the trophectoderm and the primitive endoderm develop into extra-embryonic tissues, such as the placenta.

Images courtesy of Sebastian Wennekamp, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

Box 2 | **Regulative capacity of the pre-implantation mouse embryo**

Until the blastocyst stage, the removal, addition or transplantation of cells does not impair development but can be compensated for by the adaptation of cells to their new environment. This ability of the embryo is known as regulative capacity, and it indicates that cells might undergo a form of conditional specification. Such cell specification depends on external conditions and requires that the fate of blastomeres is not yet fixed but retains a certain level of plasticity. This has been clearly shown in mice as embryos fully develop after the destruction or aggregation of cells at early stages[30,84]. Furthermore, inner cell mass cells from the mid-blastocyst stage can form functional trophectoderm derivatives[11], and trophectoderm cells can adopt an inner cell mass fate until at least the early blastocyst stage[12]. The regulative capacity of the early mammalian embryo is also exemplified by the development of twins as a result of splitting of the embryo or the inner cell mass before implantation[85,86]. Furthermore, blastomeres or embryonic stem cells injected into blastocysts are integrated into the embryo, enabling the creation of chimaeras and transgenic animals[87,88].

These three models and combinations thereof have been commonly used to interpret experiments and are thus endorsed in many articles. None of these models, however, can explain the complete set of experimental observations made regarding blastocyst patterning, in particular the complex dynamics of patterning that have been highlighted by recent imaging technologies.

New approaches and technologies

During the past decade, new experimental approaches and technologies have considerably enriched our knowledge of developmental mechanisms. Advanced live-imaging microscopy, with increasing resolution in space and time, has shown that early mammalian development is a dynamic process, with no stereotypic cleavage pattern or timing, that involves extensive cell rearrangements[7–10]. In combination with fluorescent reporter proteins, genetic manipulations and computational image analysis, it has become possible to use live-imaging microscopy to trace the fate of single cells and generate lineage trees to analyse the effects of cleavage history and cell position on cell fate[10,42–44]. The usefulness of these technologies has been demonstrated by high-resolution live imaging of transgenic embryos with a *Pdgfra*$^{H2B–GFP}$ reporter (that is, an H2B–GFP fusion gene expressed from the endogenous *Pdgfra* (platelet-derived growth factor receptor, alpha polypeptide) locus) that fluorescently marks primitive endoderm cells[35]. This study revealed the potential mechanisms resolving the mosaic of epiblast and primitive endoderm precursor cells in the inner cell mass[45] into their spatially confined pattern during blastocyst morphogenesis.

Unexpectedly, quantitative analysis at the protein level has shown that there is heterogeneity in the expression levels of key factors involved in embryogenesis in the early mouse embryo and in embryonic stem (ES) cells (reviewed in REF. 46). For example, the level of Nanog, a protein that is associated with pluripotency and specific to the pluripotent epiblast, is highly variable between cells in the early mouse embryo[33], as is also the case in undifferentiated colonies of ES cells[47]. This heterogeneity in protein levels between cells might arise stochastically by the amplification of random fluctuations in gene expression[48] or, alternatively, might represent distinct differentiation states of pluripotent cells[49]. The trophectoderm marker CDX2 is also expressed heterogeneously during pre-implantation development of the mouse embryo, although CDX2 levels do not correlate with those of Nanog[33,34].

Furthermore, quantitative analysis of mRNA expression levels using single-cell quantitative PCR or RNA sequencing can generate transcriptomic data at single-cell resolution[3,50–53]. Also, single-molecule mRNA fluorescent *in situ* hybridization (smFISH) is emerging as a suitable method to reliably quantify mRNA transcripts in embryonic cells[54–58]. These emerging techniques will enable the full characterization of molecular heterogeneity during early lineage segregation.

Finally, a recent analysis of OCT4 (also known as POU5F1) protein kinetics, using fluorescence decay after photoactivation (FDAP), indicated that differences in protein kinetics, rather than the total protein amount, might influence lineage choice[59]. This potentially adds another layer of control to mouse embryonic patterning.

Taken together, these findings reveal that mouse blastocyst patterning is both dynamic and stochastic, features that were not anticipated in the classic models.

The need for a new theoretical framework

Each of the existing models of blastocyst patterning is compatible with some of the experimental results but not with all of them. For example, no molecular determinant has yet been identified that is asymmetrically localized in the mouse zygote and can induce lineage differentiation upon induction or ectopic application, which limits support for the pre-patterning model[24–26]. The cell polarity model is missing a causal link between the apical domain, the division plane and cell fate specification. Furthermore, neither the pre-patterning model nor the cell polarity model explains the regulative capacity of the early mammalian embryo. The inside–outside model considers this regulative nature, but it is difficult to reconcile this model with results showing that cell differentiation proceeds to some extent in conditions in which the positions of the cells are mixed up or lost, for example after the isolation of blastomeres[4,60] or in the absence of cell–cell adhesion owing to the lack of E-cadherin[40]. Clearly, none of the classic models predicts the molecular heterogeneity between blastomeres identified by new techniques. Instead, they all favour a linear signalling mechanism.

To reconcile the different experimental results, it has often been suggested that these models function together or that one model is a refinement of another[61]. However, as these three classic models predict fundamentally distinct mechanisms for lineage specification, they are not easy to combine. The inside–outside model and the cell polarity model, for example, predict a different timing of the cellular events that lead to cell-lineage segregation: initiation of the progressive process of lineage specification occurs at the 16-cell stage or later in the inside–outside model compared with the 8-cell to 16-cell stage in the cell polarity model (reviewed in REF. 62).

A mechanistic understanding of the fundamental processes of mammalian embryogenesis, including symmetry breaking and lineage segregation, requires a clear theoretical framework with testable hypotheses. The three classic models leave us with an ambiguous understanding. To advance a comprehensive understanding of cellular decision making and lineage divergence in the mouse pre-implantation embryo, we think that a novel mechanistic framework is required, in particular to reconcile the high levels of molecular heterogeneity between blastomeres and the stochastic nature of the cell physiology that were recently observed.

A framework based on self-organization

The regulative capacity of the embryo, the multitude of interdependent factors that influence embryonic pattern formation and the complexities underlying the symmetry breaking process can be reconciled if the early mouse embryo is regarded as a self-organizing system (BOX 3). Indeed, recent studies showed that a reaction–diffusion

patterning mechanism mediated by Nodal and Lefty might underlie the robust generation of the embryo pattern in zebrafish[63] and of left–right asymmetry in mouse post-implantation embryos[64].

In this model, lineage establishment does not depend on a single 'master factor' but is initiated, corrected and refined by several factors. These factors may be cell characteristics (such as gene expression or cell polarity) and local interactions between cells (including chemical and mechanical inputs). In such a model, several factors induce a particular cell fate by activating the corresponding lineage-specific elements of the cellular regulatory network. Thus, the activity of a lineage-specific gene or set of genes depends on many intrinsic and extrinsic factors (FIG. 2). Each of these factors makes a specific contribution to enforcing the expression of lineage-specific genes. Lineage specification is derived from a dynamic system that is controlled by these factors, and individual cells adopt a particular fate according to the combined influence that these factors have on the core dynamics.

It is important to note that the contribution of a factor to cell fate is not binary (that is, yes or no) but can vary across a range of strengths. The strength of contribution thereby depends on the degree of presence of the factor (for example, the fraction of the cell surface that interacts with other cell surfaces), as well as on the specific temporal and spatial contexts of the cell (which progressively change according to morphogenetic events such as compaction and blastocyst cavity formation). Thus, some factors are negligible and others are dominant in certain situations, which stresses the importance of quantitative data acquisition when assessing and applying this model.

Another essential feature of this model is that factors are interconnected and interdependent, either directly or indirectly, leading to positive and negative feedback loops. This has already been demonstrated for the lineage-determining gene regulatory network that operates in mouse ES cells[65]. Furthermore, instead of restricting the framework to the gene regulatory network, we should rather consider that many non-genetic elements such as cell–cell contacts and the cytoskeleton are also part of the decision making process. These feedback mechanisms, although adding complexity to the system by giving it nonlinear properties, are important because they can provide robustness to the commitment process.

To illustrate the rationale behind this approach, we use an example in which only a few factors and interactions are considered.

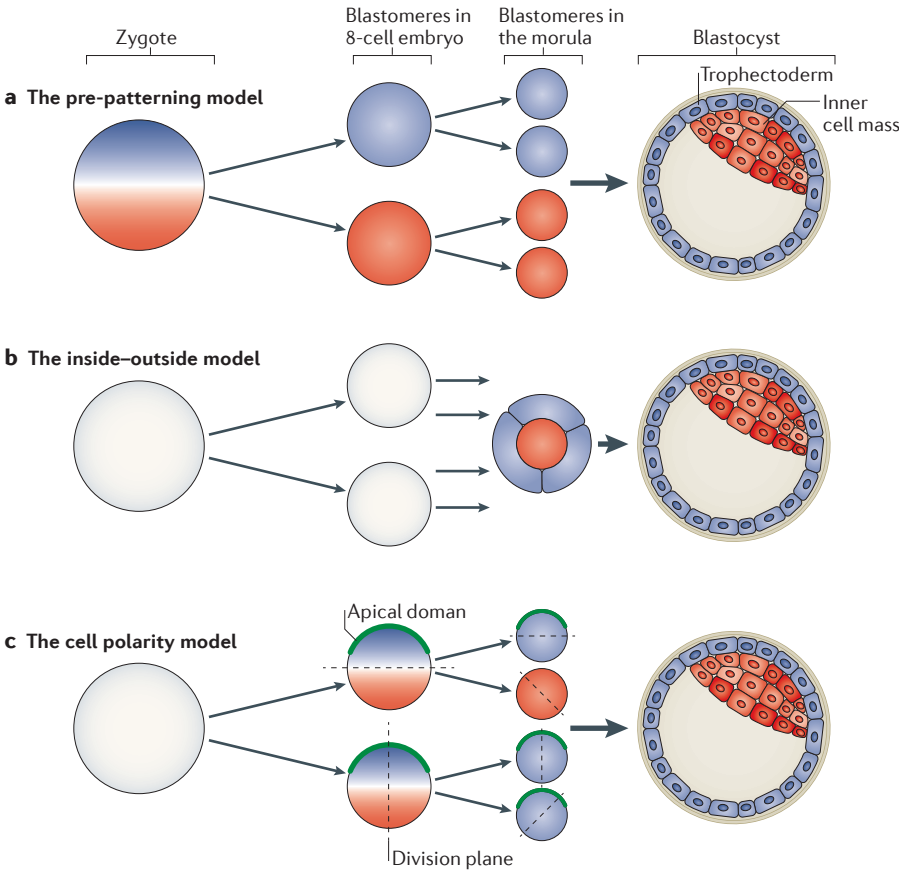


Figure 1 | **Classic models for lineage segregation in the early mouse embryo.** The figure is a gross simplification of embryogenesis and is not intended to accurately represent cell number, shape or position. Undifferentiated cells are shown in white, the trophectoderm in blue and inner cell mass cells in red. **a** | The pre-patterning model (see, for example REF. 18) proposes that the trophectoderm and inner cell mass lineages are segregated on the basis of molecular determinants that are asymmetrically localized in the oocyte (exemplified in blue and red gradients). During subsequent cleavages, these determinants are differentially segregated between daughter cells and determine cell fate. **b** | The inside–outside model[31] proposes that cell fate is induced by the position of a cell during the morula stage rather than by cell-intrinsic differences, with cells on the inside forming the inner cell mass and cells on the outside taking up a trophectoderm fate. **c** | According to the cell polarity model[4], a trophectoderm fate is induced if a cell inherits the apical membrane domain (green) after cleavage of a polarized cell (for example, of the 8-cell embryo). Asymmetric divisions (resulting from a division plane (dashed line) that separates apical and basal domains) therefore produce an inner cell mass cell and a trophectoderm cell, whereas symmetric divisions dividing the apical domain produce two trophectoderm cells.

After asymmetric division, an apolar cell in which inner cell mass-specific genes are activated could still be 'pushed' towards the outside of the embryo owing to space constraints, as the number of inside cells that are surrounded entirely by other cells is mechanically limited in an aggregate. As a result, the cell now positioned on the outside of the morula would have a decreased cell–cell contact surface area and a flatter shape[66], which might lead to the induction of trophectoderm-specific gene expression. Several factors could then influence the fate of this cell, such as cell division pattern, lineage-specific gene expression, cell–cell adhesion and cell shape. Due to its initially inner cell mass-like identity, this cell might lack polarity and have strong cell–cell adhesion and therefore would compete with other cells for a position inside the embryo. If it 'wins' this competition, the inner cell mass identity will be reinforced by surrounding cell–cell contacts. By contrast, prolonged positioning in the outer layers of the embryo would ultimately enhance the expression of trophectoderm-specific genes, which would bestow a trophectoderm identity on the cell. Which scenario will occur depends on the strength of the input of influencing factors and the specific spatial and temporal context. Importantly, these factors would inevitably cause the cell to take up an appropriate fate and position to support the formation of a blastocyst patterned with two specified cell lineages.

Box 3 | **Self-organization in biological systems**

Self-organization can be defined as the formation of complex patterned structures from units of less complexity by local internal interactions, without referring to an external blueprint or template[73]. These internal interactions typically form feedback loops, thereby conferring robustness to the system. Other common features found in self-organizing systems are nonlinearity, symmetry breaking and the emergence of patterns from stochastic fluctuations[73,89] (reviewed in REFS 90, 91). There are many examples of self-organization in biological systems.

Fate choices of multipotent stem cells
It was recently shown that the choice between self-renewal and differentiation in several pluripotent stem cell types is stochastic. In the mouse epidermis[92,93], gut[94,95] and male germ line[96], lineage tracing studies have shown that this mechanism has an important role in homeostasis, and models based on either cell-autonomous or cell-extrinsic population asymmetry have been proposed for this lineage choice event[97]. The lineage choice of these stem cell populations might share a common mechanism with the lineage choice of pluripotent cells in the early mammalian embryo.

***Arabidopsis thaliana* shoot apical meristem**
The shoot apical meristem of *A. thaliana* exhibits self-organization, and a mechanism for pattern formation during organogenesis that seems to include mechanical and biochemical inputs has been described. Mathematical models have recently been proposed to explain the observed patterns by combining these different inputs and also introducing feedback loops[98,99]. These models might serve as a template for modelling approaches in the early embryo that integrate mechanical and biochemical factors.

***Dictyostelium discoideum* migratory slug and fruiting body formation**
Upon starvation, the unicellular amoeba *D. discoideum* forms cell aggregates by moving towards waves of cyclic AMP (cAMP) that are propagated from cell to cell. This process coincides with a change in the adhesive properties of *D. discoideum*[100]. It has been shown that this self-organizing process of slug formation can be modelled by considering only cAMP production, chemotaxis and cell adhesion[101,102]. By adding cell differentiation and rigidity to the model, but keeping all other parameters constant, it is possible to simulate the complex morphogenesis of the fruiting body by a multiscale model[103]. Interestingly, the spatial pattern of this self-organization over a long range is fine-tuned by a genetic feedback mechanism[104].

Features of the self-organization framework. The stochastic self-organization theory provides a new conceptual framework to understand blastocyst patterning by applying the principles of self-organization to early mammalian development. It integrates many of the factors that influence lineage segregation and thus could reconcile the experimental findings in normal and experimental conditions that seem to be contradictory to the classic models.

The mechanism of cell lineage segregation in the self-organization theory encompasses intrinsic factors (as do the cell polarity and pre-patterning models) and extrinsic factors that feed back on cell fate to account for the highly regulative nature of the embryo (as proposed in the inside–outside model). It may thus be regarded as a reformulation of the ideas underlying the classic models. The new theory, however, will also have to explain recent findings, such as heterogeneity in the expression of key transcription factors, that are not compatible with any of the classic models. We believe that the self-organization theory is a comprehensive approach to explain all observed phenomena in a quantitative manner.

Importantly, the acting factors to be considered in the theory are not all deterministic; for example, spindle alignment can be random and gene expression can be 'noisy'. Stochastic fluctuations, which are an intrinsic feature of any biological system, can nevertheless lead to binary decisions, as shown, for example, in a synthetic yeast-based system using a short-lived, stochastically expressed transcription factor with multiple binding sites at its own promoter[67]. Also, in multipotent mouse haematopoietic progenitor cells, fluctuating gene expression can direct the stochastic priming of lineage specification[68]. However, such fluctuations in a self-organizing system imply that, in some cases, cells in a distinct part of the embryo could potentially make incompatible fate decisions downstream of stochastic gene expression owing to the lack of direct cell–cell interactions. This might result in apoptosis of these cells, as observed in the late blastocyst[35,69].

Considering the stochastic fluctuation present at the molecular level, cell fate should be considered as a statistical outcome rather than a simple binary decision. Given a certain spatial and temporal context of the embryo, some bias in the pattern of cell division or lineage specification might become discernible. However, this by itself does not substantiate a mechanism relying on the segregation of determinants. For example, the pre-patterning model might be able to explain a fraction of the embryos exhibiting this bias, but the integration of stochastic fluctuations with self-organizing properties could explain possible biases in all embryos.

This framework might also explain why the symmetry-breaking 'cue' remains to be identified. In a self-organizing system, a dominant 'master factor' is not required, and symmetry breaking can be initiated by a stochastic disturbance of the system, which is then amplified through a feedback loop. The feedback loop that is triggered might not always be the same, and the relative importance of different feedback routes could vary as a function of the developmental stage or the specific environment of the cell.

Modelling self-organization

A self-organizing system depends on multiple interdependent factors and feedback loops in a certain spatial and temporal context, including intrinsic fluctuations. Defining such a system requires the quantitative acquisition of parameter values and computational modelling (Supplementary information S1 (box)). The model should then be able to predict the cellular behaviour of the developing embryo *in vivo* and under experimental conditions, which can be tested experimentally.

Building a computational model. Developing a theory that accounts for the factors that influence the activity of a gene regulatory network and therefore cell lineage choice requires the quantitative characterization of each factor independently. To this end, it will be essential to determine the experimental settings in which such factors can be separated and independently assessed. This can, for example, be achieved by isolating blastomeres to analyse their behaviour and gene expression response to experimental manipulations of cell shape or polarity in the absence of cell–cell interactions. In principle, a rigorous model of the cellular process could be constructed from the acquired quantitative data. To evaluate the resulting model, it will be necessary to observe *in vivo* embryonic development at the single-cell level combined with a live readout of gene expression dynamics. The development of fluorescently tagged reporters to measure the activity of lineage-specific genes[35,70], together with advanced microscopy[71,72], should allow the quantitative acquisition of this information at high resolution in space and time. In addition, single-cell transcriptome analysis[3,50–53] can provide

comprehensive and quantitative expression data at the RNA level that are suitable for statistical analysis of the behaviour of the cell population as a whole. The model and its predictions can then be refined in a cycle of theoretical simulations and *in vivo* experiments[73]. Importantly, the statistical distributions of stochastically fluctuating cellular variables have to be measured and predicted. It will be equally important to model experimental perturbations (for example, gene induction, gene knockout or physical constraints), such that the model can be tested thoroughly.

Given the painstaking measurement of parameters that is required for modelling, what could be the benefit of considering self-organization in experimental biology today?

A mechanical reference model. Perhaps the first information to be gained from a self-organization theory is a prediction of how mechanical constraints and physical properties alone determine embryonic morphogenesis during the first six rounds of division after fertilization, to form 64 cells and a blastocyst cavity. During this period, the embryo is sealed from the outside by the cell–cell junctions of the outermost epithelial cell layer and has essentially the same total cell volume as the zygote. In other words, the model should represent the hypothetical situation in which blastocyst morphogenesis arises solely from physical processes, ignoring genetic activity altogether but considering volume constraints, membrane elasticity and other physical elements. In this scenario, differential gene expression is an outcome. Once this hypothetical model is established, it will serve as a reference, and by comparing this model with cellular morphogenesis occurring *in vivo* one would be able to estimate the contribution of the 'cellular chemistry' to this process. The disparity of a specific physiological trait *in vivo* would clearly indicate that another factor is required in addition to physical processes. In this way, it would be reasonable to add the contribution of asymmetrically localized molecules to the model or simply early differentiation in the expression profiles of different cells.

Such a baseline for blastocyst morphogenesis that incorporates the physical aspects of the process has never been fully established nor formally tested against experimental results. This would be desirable, as the lack of a theoretical framework might lead to an incorrect interpretation of experimental data. For example, certain skewed patterns of cell division in the pre-implantation embryo might be an outcome of physical constraints imposed on the cells or of the spindle being oriented by proteins localized to specific subcellular regions. This could be critically evaluated by comparing experimental data on spindle orientation in the embryo with the prediction of the reference model based only on cell shape. The measurement of spindle orientation in isolated cells that are forced to adopt a specific cell shape could then be used to test whether cell shape is sufficient to predict spindle orientation in an embryonic cell. The lack of such a reference model might underlie a long-standing controversy in the field about the presence or absence of cell fate determinants[24,29]. The importance of physical constraints has already been discussed in early attempts at computer simulation of blastocyst morphogenesis[66,74,75]. However, these simulations lacked predictive power owing to the insufficient measurement of cellular parameters.

In the long term, the strategy described here may lead to a comprehensive understanding of early mammalian development, eventually integrating the multiple factors that influence cell fate at multiple levels.

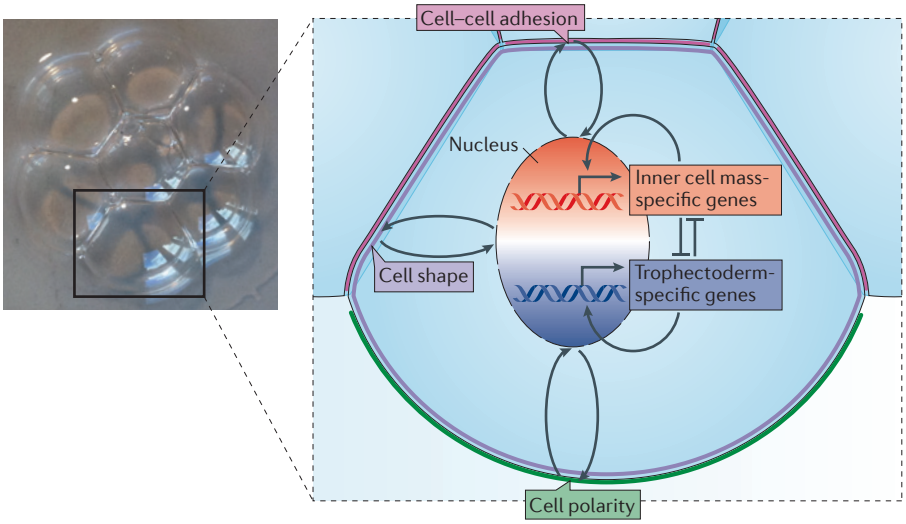


Figure 2 | **The self-organization theory of early mammalian development.** The self-organization theory encompasses the physical properties of the cell, as well as differential gene expression patterns and the localization of molecules. The physical properties of cells in the morula are probably comparable to those of the depicted soap bubbles, which self-assemble into a reproducible structure determined by cell adhesion and surface tension[105]. In addition to these physical properties, cells in the early mouse embryo have molecular components that generate lineage-specific gene expression patterns and polarity, as shown in the inset. The physical properties and characteristics of cells, such as shape, cell–cell adhesion and polarity domains, can influence the activity of gene expression (inner cell mass-specific gene expression is depicted in red and trophectoderm-specific gene expression in blue). The reverse is also true, as gene expression can modify the physical parameters of a cell, such as cortical tension (which determines cell shape) and cell–cell adhesion, thereby influencing the position of a cell in the aggregate to fit its gene expression profile. Feedback loops can stabilize the state of a cell, or they can amplify a small difference in gene expression or physical property and thereby significantly alter the characteristics of a cell, causing it to compete for a position suited to its properties. Finally, stochastic fluctuation of factors, for example of gene expression, can introduce additional dynamics. Altogether, the state of the cell is determined by the interaction between intrinsic and extrinsic factors, and this state dynamically changes during morphogenesis. Cells will progressively evolve to eventually make a binary decision (such as commitment to either an inner cell mass or trophectoderm fate in the blastocyst), thereby establishing the reproducible blastocyst pattern observed in embryos. Image courtesy of Sebastian Wennekamp, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

Open questions and possible approaches

Although recent progress in mouse molecular genetics has led to the identification of essential molecules and signalling pathways that function in early development[32–34,43,45,65,76–83], several fundamental questions regarding early mammalian development still remain to be explored.

First, given the 'noisy' environment of embryogenesis with random fluctuations of gene expression, how might it be possible to experimentally detect and define the onset of symmetry breaking? The establishment of comprehensive blastomere lineage maps, by tracking cell position, cell shape and gene expression profiles over time using fluorescent reporters, and their full statistical analysis — including comparison of the size, distribution and composition of the clonal descendants of blastomeres at the 2-cell,

4-cell, 8-cell and 16-cell stages — will be invaluable. This will allow us to identify the time point of symmetry breaking at which clones change their characteristics and form distinct clusters.

One could then investigate the mechanism by which symmetry is broken in the embryo. Given the number of factors that might function in blastocyst patterning and the possibility that small differences could be amplified by feedback loops, it would be crucial to study experimental systems in which the number of acting factors and their interactions are minimized to identify the first differences and ask which of these factors is sufficient for breaking symmetry.

Another remaining key question concerns the mechanism by which the embryo translates the process of symmetry breaking into the final outcome of lineage restriction to form a blastocyst with a reproducible pattern, despite the stochastic cell-to-cell and embryo-to-embryo variability in terms of the pattern of cell division and initial gene expression. The recent live imaging of transgenic embryos with a *Pdgfra*$^{H2B-GFP}$ reporter that marks the primitive endoderm lineage[35] demonstrated that both cell sorting and adjustment of gene expression according to cell position within the embryo can account for the resolution of stochastic heterogeneity into reproducible patterns. This is in line with the idea that a cell in the developing blastocyst recognizes its position in the embryo (that is, inside or outside). However, the identity of such positional information or the cues that cells can use to recognize their position remain elusive. Possible cues include the extent of cell–cell interactions or the presence of a cell-free surface[32], environmental cues (such as differences in metabolic activity between inside and outside cells)[31]] or cell mechanics (such as cell volume, shape or cortical tension)[66]. Again, it would be crucial to use experimental systems in which the function of each of these potential cues can be assessed independently.

The self-organization theory as discussed here offers a new framework in which we can integrate molecular and physical parameters to address these remaining questions regarding blastocyst morphogenesis and patterning.

Conclusions

The mechanism underlying symmetry breaking in mammalian embryos that leads to the formation of molecularly and spatially coordinated cell lineages is elusive. The current models — although offering important ideas — do not explain all features of early mouse development. Recent approaches and new technologies have shed further light on the dynamic and complex nature of this process, and the new findings are not in agreement with any of the classic models of early mouse patterning in isolation. We propose to use the principle of self-organization to explain blastocyst patterning and morphogenesis at the systems level. As it can integrate intrinsic and extrinsic chemical and physical factors acting on individual cells in a spatial and temporal context, this approach might enable a comprehensive understanding of mammalian symmetry breaking and blastocyst development. To this end, it will be essential to identify the key factors that influence symmetry breaking and to evaluate them independently and quantitatively. Experimentalists should record the statistical distribution of the possible outcomes, and theoreticians should refine their theories to make interesting predictions about the system.

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Competing interests statement

The authors declare no competing financial interests.

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